The Riddle of Rhamnolipid Molecular Biosynthesis

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Rhamnolipids (RLs) are the best characterised glycolipid biosurfactants. Rhamnolipid biosynthesis is actioned by three enzymes which were first discovered in the classic RL producing bacterium *Pseudomonas aeruginosa,* **fig 1**.

RhlA – produces fatty acid dimers by intercepting intermediates in the de novo fatty acid synthesis pathway (1).

RhlB – Conjugates a single rhamnose moiety onto the fatty acid dimers synthesised by RhlA forming mono-RLs (2)

RhlC – Using mono-RL as a substrate conjugates a second rhamnose moiety forming di-RLs (3)

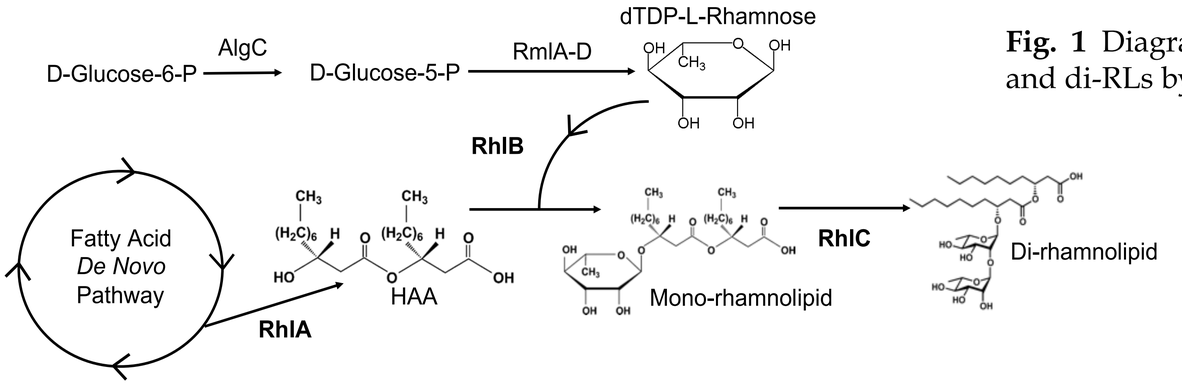

Fig. 1 Diagram depicting the biosynthesis of both mono and di-RLs by enzymes RhlA, RhlB and RhlC (1-3)

The enzymes involved with RL biosynthesis are encoded by three genes; *rhlA*, *rhlB* and *rhlC*. Orthologues of these gene have been identified in a number of species of bacteria belonging to the *Pseudomonas* & *Burkholderia* genera (4,5). The arrangement of these genes can however be dramatically different depending on species, **fig 2**.

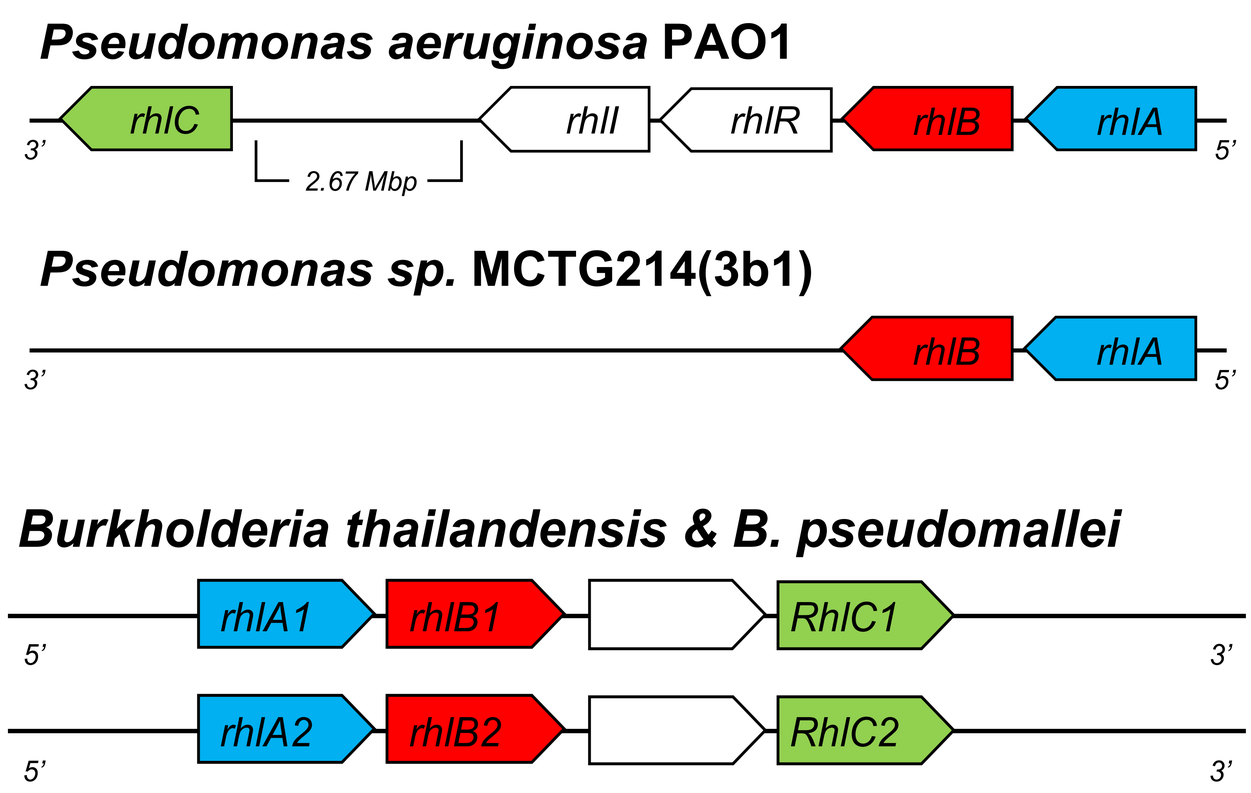

Fig. 2 The genomic arrangement of RL biosynthesis genes in four RL producing species belonging to the *Pseudomonas* and *Burkholderia* genera. Diagram generated based on data provided in *Pseudomonas* and *Burkholderia* genome databases (6,7).

The first difference between the RL synthesis genes in *Burkholderia* species compared to that in *P. aeruginosa* is that *Burkholderia* possesses two identical and functional copies of each gene which are located separately on the chromosome, **fig 2** (6,7).

A second key difference is the level of sequence homology of these two genera. At both the genetic and amino acid levels the two genera only share approx. 40% homology. This sequence homology rises significantly when you compare RL producing species belonging to the same genus i.e *B. thailandensis* and *B. pseudomallei*.

These differences in both genomic arrangement and sequence homology may indicate early divergence in RL biosynthesis evolution and could also account for the differences in RL congener profiles observed in these two genera.

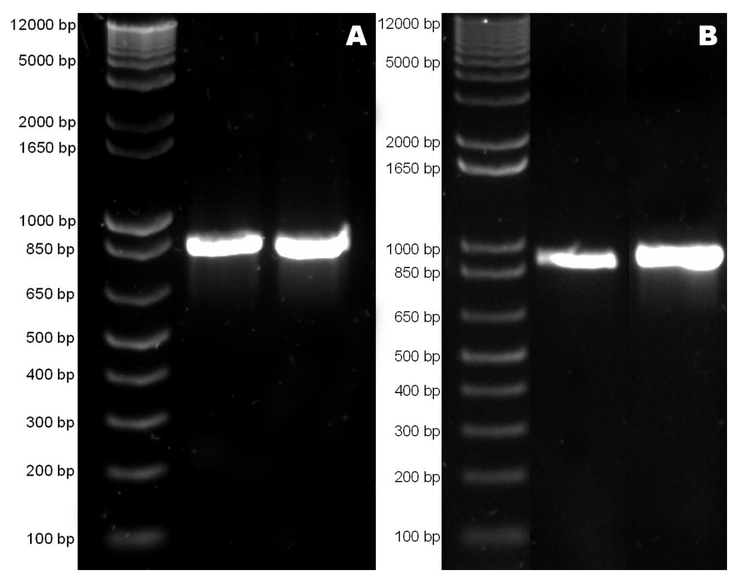

Fig. 3 PCR amplification using primers for *rhlA* (**A**) and *rhlB* (**B**) and genomic DNA extracted from *Pseudomonas sp.* MCTG214(3b1) and *P. aeruginosa* PAO1 resulted in amplicons of matching size. Subsequent DNA sequencing showed the MCTG214(3b1) amplicons to posses 99% identity to *rhlA* and *rhlB* of *P. aeruginosa* (8).

We recently identified a marine bacterium phylogenetically similar to *Pseudomonas mendocina* with the ability to synthesise RLs. Using primers based on the sequences of other RL producing *Pseudomonas* strains we amplified DNA fragments matching the size of *rhlA* and *rhlB*, **fig 3** (8). DNA sequencing showed these genes to be 99% homologous to *rhlA* and *rhlB* of *P. aeruginosa* and genomic sequencing showed these genes to be located together in the chromosome, **fig 2** (8). Interestingly our PCR screening **did not identify any *rhlC* ortholog in this strain presenting a paradox on how this strain synthesises the di-RLs observed in chemical analysis of culture extracts (8). Based on our genetic analysis we postulate that this strain may have obtained its RL synthesis genes via horizontal gene transfer and possesses an as yet unidentified gene responsible for di-RL synthesis.**

We have also identified RL production in another marine bacterial strain belonging to the genus Marinobacter. PCR screening using primers for both the *Pseudomonas* and *Burkholderia rhl* genes have not yielded any amplification products. We therefore predict that RL synthesis is being carried out either by a novel biochemical pathway or, more likely, by RL synthases structurally different from those already elucidated. Therefore we have developed and are employing a strategy for the detection of novel RL synthases and the genes encoding them, **fig 4**.

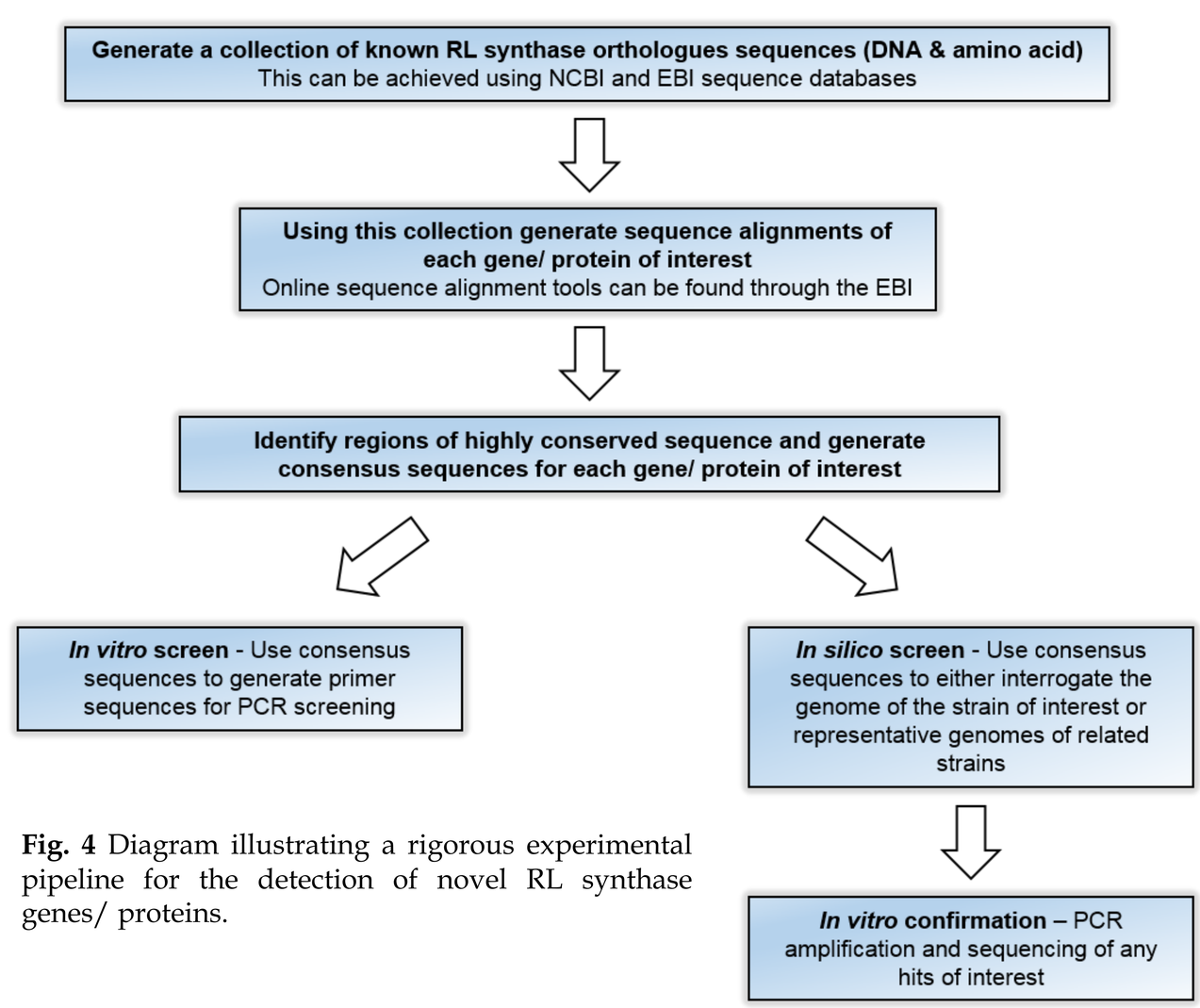

Fig. 4 Diagram illustrating a rigorous experimental pipeline for the detection of novel RL synthase genes/ proteins.

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